ANTIOXIDANT STATUS IN DAIRY COWS DURING LACTATION

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Abstract: During the transition period important metabolic changes occur in dairy cows, which can also experience oxidative stress. The study was carried out to assess the activity of antioxidant enzymes, the level of lipid peroxidation and the level of serum antioxidants, carotenoids and uric acid. SOD activity in cows was situated around 1365 U/g Hb in the first week after parturition, 1890 U/g Hb in the second week, 2290 U/g Hb in the third week, 1896 U/g Hb in the fourth week, and 1905 U/g Hb in the sixth week after parturition, value close to that of the cows in late lactation (2005 U/g Hb). GPX activity in cows was situated around 129 U/g Hb in the first week after parturition, and than increased to reach 166 U/g Hb in the sixth week after parturition, value comparable with that of cows in late lactation (160 U/g Hb). The same pattern of enzyme activity modification was observed for blood catalase. The maximum level of lipid peroxidation was observed in the first week after parturition (62 μmols/l), comparing with the level of MDA in the cows in late lactation (35 μmol/l). In the second week after parturition the level of MDA decreased significantly, and remains at approximately same values during the experiment. The high level of MDA in the first week after parturition correlates with a low activity of antioxidant enzymes (SOD, GPX, catalase) and a low level of antioxidants. The experimental data show that during parturition and onset of lactation oxidative stress appears in dairy cows, but these modifications are of short-therm.

INTRODUCTION

Parturition and the onset of lactation represent periods of drastic physiological changes and intense metabolic demands in dairy cows. Important changes occur during parturition and early lactation, especially in the lipids and proteins metabolism [Marcos et al., 1990; Grum et al., 1996; Minor et al., 1998, Bell, 1995; Drackley, 1999; Doepel et al., 2002; Park, et al., 2002; Turk et al., 2004; Castillo, 2006]. The transition period is under strict hormonal control, which coordinate the activity of all tissues and organs in order to respond to this metabolic demands [Castillo et al, 2005; Vernon, 2002; Castillo et al. 2006]. The intense metabolic processes are accompanied by modification of energetic metabolism and by an increase of oxygen consumption. This lead to an increase in the generation of reactive oxygen species (ROS), which are normally neutralized by enzymatic and non-enzymatic defense systems of living organisms. The imbalance between the rate of ROS production and their neutralization lead to the oxidative stress [Frei et al., 1988; Sies, 1991; Trevisan, 2001]. It was shown that during transition period, oxidative stress can occur and it can contribute to some periparturient disorders or metabolic diseases [Miller and Madsen, 1994; Brezezinska-Slebodzinska et al., 1994; Ronchi et al., 2002; Bernabucci et al, 2002; Bernabucci et al, 2005].

The aim of this study was the evaluation of antioxidant status during early lactation period, first 6 weeks after parturition, comparing with late lactation. The activity of main antioxidant enzymes: superoxiddismutase (SOD), erythrocye glutathion peroxidase (GPX), catalase was determined, together with the level of lipid peroxidation level (malondialdehyde...
assay MDA). Uric acid and carotenoids, known as non-enzymatic antioxidants, were also determined in serum [Hicks et al., 1993; Krinsky, 1994].

MATERIALS AND METHODS

The experiment was carried in U.S.A.M.V Cluj-Napoca farm, on a group of ten cows Baltata Romaneasca, in a period of six weeks (end of March – May). All the cows gave birth in the same period and were fed with the same diet during the experiment.

SOD was determined in the erythrocytes lysate using the kit RANSOD, from Randox Laboratories. It is based on the inhibition of I.N.T. (a tetrazolium salt) oxidation by superoxid anion generated in xanthine/xanthine oxidase system., in the presence of blood SOD.

GPX activity assay is based on the method of Paglia and Valentine and uses the Ransel kit from Randox Laboratories. For both enzymes the hemoglobin content in blood was measured and the activity expressed as Units/g Hemoglobin.

Catalase activity was determined by a titrimetric method, using potassium permanganate.

The level of lipid peroxidation was determined using the spetrophotometric assay with thiobarbituric acid.

Total carotenoids were estimated by spectrophotometric assay, at 450 nm, of an hexanic extract obtained from plasma.

Uric acid was determined using an enzymatic method with uricase, provided by Hospitex Diagnostic.

RESULTS AND DISCUSSIONS

Superoxiddismutase (SOD) accelerates the dismutation of the toxic superoxide radical to hydrogen peroxide and is considered the first intracellular defense against reactive oxygen species. The cytosol of all eukaryotic cells contains CuZn-SOD. Determination of SOD is important in the evaluation of antioxidant status, under physiological or pathological conditions. As can be seen in Fig. 1, the SOD activity has an average of 2045 U/g Hb for cows in late lactation. A significant decrease of SOD activity can be observed in dairy cows in the first week after parturition. The activity increased starting with the second week, and was close to the control after six weeks.

Glutathione peroxidase (GPX) plays an important role in cellular antioxidant defense. It uses glutathione as reducing agent and reduces hydrogen peroxide and other peroxides to water, or respectively to the corresponding alcohol. Determination of GPX activity might be beneficial in the evaluation of selenium status and in the evaluation of antioxidant status. For the cows in late lactation the average GPX activity was situated around 169 U/g Hb. One week after parturition a decrease of GPX activity was observed, followed by a significant increase in the second week, and a normalization after six weeks (Fig. 2). The high values of GPX and SOD in the second, and respectively in the third week can be interpretate as an effort of the organism to adapt to a high level of ROS production. The same pattern of enzyme activity modification was observed for blood catalase.
Malondyaldehyde (MDA) is considered the final product of lipid peroxidation and a marker of oxidative stress. As can be observed in the Table 1, the maximum level of lipid peroxidation was observed in the first week after parturition (62 µmols/l), comparing with the level of MDA in the cows in late lactation (35 µmol/l). The total lipid serum concentration is also lower in the first week, and this is explained by an intense lipolysis and mobilization of fatty acids in order to sustain the lactogenesis. A similar pattern was observed in recent studies regarding MDA and TAS (total antioxidant status) in dairy cows [Castillo et al., 2005, Castillo et al., 2006]. The modification is of short-therm, since in the second week after parturition the level of MDA decrease significantly, and remains at approximately same values during the experiment. The high level of MDA in the first week after parturition correlates with a low activity of antioxidant enzymes (SOD, GPX, catalase). Total carotenoids and uric acid concentration in serum were also low in the first week after parturition (Table 1). All these data show that the parturition and onset of lactation can induce, beside the important modification metabolic status, an oxidative stress.
Fig. 2. Activity of erythrocyte glutathione peroxidase (GPX) during lactation

Fig. 3. Serum MDA level during lactation
Table 1. Concentration of total lipids, MDA, carotenoids and uric acid during lactation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total lipids mg/100 ml</th>
<th>Total carotenoids µg/100 ml</th>
<th>Uric acid mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late lactation</td>
<td>500.6±28</td>
<td>242.5±22</td>
<td>1.09±0.08</td>
</tr>
<tr>
<td>Week 1</td>
<td>146.2±17</td>
<td>105.8±15</td>
<td>0.57±0.06</td>
</tr>
<tr>
<td>Week 2</td>
<td>212.5±18</td>
<td>144.8±17</td>
<td>1.47±0.09</td>
</tr>
<tr>
<td>Week 3</td>
<td>438.0±24</td>
<td>155.2±16</td>
<td>0.99±0.08</td>
</tr>
<tr>
<td>Week 4</td>
<td>354.7±21</td>
<td>257.3±27</td>
<td>0.93±0.07</td>
</tr>
<tr>
<td>Week 6</td>
<td>506.2±31</td>
<td>279.8±30</td>
<td>1.23±0.10</td>
</tr>
</tbody>
</table>

We have to remark that the inter-individual variations on MDA values, as well as for enzyme activities are rather high and this can be linked to the adaptative capacity of each organism, in a order to optimize the consumption of oxygen and neutralization of free radicals.

CONCLUSIONS

Besides metabolic parameters, the antioxidant status in dairy cows changes during parturition and early lactation. The activity of antioxidant enzymes: SOD, GPX, catalase is lower in first week after parturition and increase afterward, reaching the normal value for dairy cows in six weeks. It correlates with low level of serum antioxidants: carotenoids and uric acid, and with a high level of lipid peroxidation (MDA concentration).

The experimental data show that during parturition and onset of lactation oxidative stress appears in dairy cows, but these modifications are of short term.

BIBLIOGRAPHY